EMBRYONIC DETERMINATION IN THE ANNELID, SABELLARIA VULGARIS

II. Transplantation of Polar Lobes and Blastomeres as a Test of their Inducing Capacities

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Introduction

E. B. Wilson (1904*a*) demonstrated that the egg of the annelid, *Lanice*, belonged to the group of so-called mosaic eggs, since isolated halves of the two-cell stage developed into partial embryos. The anterior cell produced an embryo which lacked the post-trochal region; in the embryo formed from the posterior cell there was present a nearly typical post-trochal region. Delage (1899) had previously described a dwarf embryo from an egg-fragment of the same species. That annelid eggs, generally, are of the mosaic type is shown by the experiments of Penners (1924, 1926) with *Tubifex*, of Tyler (1930) with *Chætopterus*, and of Hatt (1932) with *Sabellaria*.

Wilson (1929) summarizes the evidence which indicates that there is no fundamental distinction between the mosaic and regulative types of ova. Among regulative eggs, where correlative differentiation, or embryonic induction, is most prominent, mosaic features can be found, and in mosaic eggs, there are suggestions that embryonic induction may play a part in early development. Wilson suggests that the polar lobe of such eggs as *Dentalium* may function as an organizing region similar to the dorsal blastoporal lip of amphibia, since only when the lobe is present does the larva develop the apical tuft and the post-trochal region. However, in the absence of transplantation experiments, no final conclusions could be reached.

Schleip (1929) describes a "natural experiment" in which a second polar lobe is added to the egg of *Dentalium*. Among giant eggs, some are found which appear to be fusions of two ova at their vegetal hemispheres. In these eggs, a single large polar lobe may be formed, which goes in its entirety into one of the cells. This leaves one egg with no lobe, and the other with two. However, such eggs do not develop. Schleip then tried to transplant isolated polar lobes to blastomeres, but all attempts were unsuccessful.

Transplantation experiments can readily be performed in the egg of *Sabellaria vulgaris*. In this species, large polar lobes, similar to those found in *Dentalium*, are formed in the course of the first three cleavages. Although the egg is only about sixty micra in diameter, it is not difficult to remove the polar lobe or to separate individual blastomeres. Both blastomeres and polar lobes can be fused together in desired combinations and the eggs reared through a well-developed trochophore stage. In all, 247 successful transplant operations have been studied, including 80 in which either the first or second polar lobe was transplanted.

Material and Method

All experiments reported in this paper were performed at the Marine Biological Laboratory, Woods Hole, Mass., during the summers of 1935, 1936, and 1937. The animals used were dredged from Vineyard Sound and the eggs were obtained in the manner described elsewhere by the writer (1937). The egg of *Sabellaria vulgaris* possesses a tough, wrinkled vitelline membrane which resists cutting with glass needles. In addition, within the membrane, in the perivitelline space, there is a dense jelly which makes difficult the separation of individual blastomeres and which completely prevents bringing blastomeres together. Thus, to perform transplantations, it is necessary to first remove both membrane and jelly from the egg.

The vitelline membrane is removed from fertilized eggs of *Sabellaria* by treatment with an isotonic solution of NaCl, brought to pH 9.6 by the addition of Na_2CO_3 (Novikoff, 1938). In most cases, the eggs are thus treated within the ten minutes that elapse between the formation of the first polar body and the second. After they have been washed once in sea water, the denuded eggs are placed into a Syracuse dish of freshly-filtered sea water. The dish is allowed to remain without disturbance on the stage of a dissecting microscope. Within two or three minutes, the eggs have settled and are adhering to the glass bottom of the dish. By means of fine glass needles, each egg is then lifted from the jelly which remains adherent to the bottom. This process may have to be repeated several times in order to remove the jelly completely. The jelly being invisible, its removal can best be ascertained by bringing together the individual eggs; they come into contact with each other only when the jelly has been removed. At this time the eggs are quite sticky and if allowed to adhere too long to the dish they flatten out. Since such flattened eggs do not develop normally, it is important that the eggs be lifted, at close intervals, from the bottom of the dish.

The eggs are cut, free-hand, under the dissecting microscope. In order to determine the orientations of cells when fused, small spots are marked on the eggs before they are cut. This is done by bringing into contact with the surface of the egg the open end of a fine capillary tube filled with agar, in which is dissolved a vital dye such as Nile Blue sulphate. In order to fill an exceedingly fine bore, the following procedure is followed. A short piece of capillary tubing is partly filled by immersing one end in a warm solution of the agar containing the dye. When the agar has cooled, that part of the tube which has no agar is heated by a microflame and pulled out to the desired width of bore. The other end of the tube is then sealed off and the microflame applied to the part of the agar nearer the narrowed end. The agar melts and moves into the free end of the tube. On cooling, the agar, in many cases, remains at the opening of the tube. The tube is brought into contact with the egg by means of a Zeiss- Peterfi micromanipulator. The length of time during which the agar must remain in contact with the egg varies with the concentration of dye used. In some cases, where it is possible to determine the polarity of the isolated cell without previously staining a particular region, the entire cell is stained before transplantation. This is usually done before the membrane is removed, and a dilute solution of Nile Blue sulphate in sea water is used.

To effect the fusion of blastomeres it is only necessary to bring them into contact with each other, after the membrane and jelly have been removed. It is usually sufficient to press them together for several seconds, although this may, in some cases, have to be repeated several times before they finally stay together.

Following the operation, the eggs generally develop into swimming larvæ within five to six hours after fertilization. They can at that time no longer be left in the open dish for they soon swim to the surface, where they are quickly torn by the surface film. The procedure which leaves the least amount of surface exposed to the air and which, at the same time, is most convenient for the detailed study of the living larvæ, is to allow the embryos to develop in small drops of sea water between a glass slide and coverslip. When an embryo begins to show signs of movement, it is transferred, by means of a mouth pipette (Hörstadius, 1937*a*), to a small drop of sea water. Evaporation from the drop is prevented by sealing the edges of the coverslip with a thin layer of vaseline.

The results to be described are based on a total of 403 experimental larvæ, 156 developing from isolated blastomeres and 247 from fusions of various blastomeres. The percentages of larvæ surviving are: 73

per cent on the first day, and 51 per cent on the second day, from isolated cells; 91 per cent on the first day, and 74 per cent on the second day, from combinations of blastomeres. As described in the first paper of the present series (Novikoff, 1938), a great number of the larvæ arising from denuded eggs develop into exogastrulæ, in which there is no internal gut and the endoderm cells are turned inside out. Under the coverslips, all structures differentiate in the larvæ, just as they would in larger volumes of sea water, with the exception of the orange-red eye spot, and, to some extent, the yellow-green chromatophores. These are frequently absent or irregular. That the failure of the eye spot and the chromatophores to develop normally is due to some general factor such as lowered oxygen tension or increased pressure, is indicated by the development of normal eggs (i.e., eggs from which the membranes have not been removed) under similar conditions. Although the cell arrangement and the tissues of the larvæ from such eggs are manifestly similar to those of normal larvæ, the eye spot does not form and the chromatophores show the same variable character as do those of the membraneless larvæ.

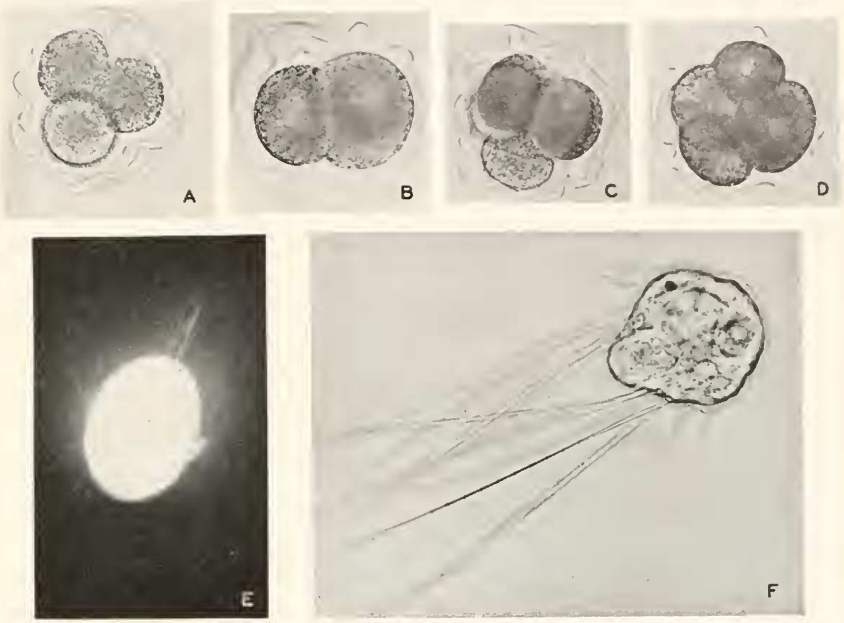
Normal Development

Changes in the egg of *Sabellaria vulgaris* at maturation and during fertilization are described elsewhere (Novikoff, in press). Both polar bodies are formed after insemination and cleavage begins approximately twenty minutes [1] after the extrusion of the second polar body.

In the course of the first cleavage division, a large part of the vegetal hemisphere of the egg becomes constricted in the form of a spherical lobe. Figure 1, *A* is a photograph of an egg at the "trefoil" stage, when the polar lobe is at its maximum size. When viewed from the side, the lobe appears to be equal in size to either of the first two blastomeres. But when viewed from the vegetal pole, it is seen to be considerably smaller. The visible constituents of the lobe cytoplasm do not differ from that of either blastomere, except that there is no spindle area in the lobe. About fifteen minutes after it first appears, the polar lobe flows into one of the blastomeres. This blastomere is the *CD* cell and it is now much larger than the other, the *AB* blastomere (Fig. 1, *B*). Figure 1, *C* shows an egg during the second cleavage, when the second polar lobe is at its maximum size. This lobe forms in the *CD* blastomere only, and is smaller than the first lobe. When it flows back into one of the daughter cells at the completion of the division, the four quarter-blastomeres consist of two equal-sized cells, *A* and *B* (the products of the division of *AB*), a slightly larger cell, *C*,

[1] All time intervals are for room temperatures, varying from 19° to 25° C.

and a much larger cell, *D* (Fig. 1, *D*). It is the *D* blastomere which has received the contents of the second polar lobe. During the next division, when the micromeres are produced, a third polar lobe, formed



FIGS. 1–10. All figures, except 1 and 5, are composed of camera lucida drawings of living larvæ, magnified approximately 260 times. Abbreviations used are:

p	prototroch
at	apical tuft
ac	apical cilia
ec	cilia of exogastrulated endoderm cells
pc	posterior cilium
dc	dorsal cilium
pb	post-trochal bristle
e	eye
m	mouth

FIG. 1. Photomicrographs of living eggs of *Sabellaria vulgaris*. *A*. Trefoil stage. Side view. The polar lobe is slightly out of focus. *B*. Two-cell stage. Blastomere *CD* is to the right of *AB*. *C*. Second cleavage. The second polar lobe and two of the quarter-blastomeres are in sharp focus; the other two cells are not in focus. *D*. Four-cell stage. Seen from the vegetal pole. The large *D* cell is to the right and the *A* cell is uppermost. *E*. Early trochophore larva. Sixteen hours after fertilization. Photographed with dark-field illumination; shows apical tuft and prototrochal cilia. *F*. Later trochophore larva. Forty-eight hours after fertilization; shows stomach, intestine, dark eye spot, prototrochal cilia, and post-trochal bristles.

from the *D* cell, flows into the *D* macromere, 1*D*. This lobe is smaller than the second lobe and is more variable than the preceding lobes;

in many cases this lobe does not become distinctly separated from the dividing *D* cell. The later cleavages of the egg have not been described.

A detailed description of the development of the larva is presented in the first paper of this series (Novikoff, 1938). In the normal course of development, the ectoderm gives rise to the following structures: a prototroch of long active cilia; chromatophores; an apical tuft; non-motile apical cilia; paired chætæ-sacs from which extend long, serrated bristles; a single posterior non-motile cilium; several long non-motile cilia on the dorsal surface; an eye spot; and a neurotroch of rapidly moving cilia. The endoderm differentiates into a tripartite gut consisting of œsophagus, stomach, and intestine. In many of the membraneless larvæ, the gut is exogastrulate and the three portions can not be distinguished. Of the ectodermal structures, those most easily observed in living embryos—and therefore the ones best suited for

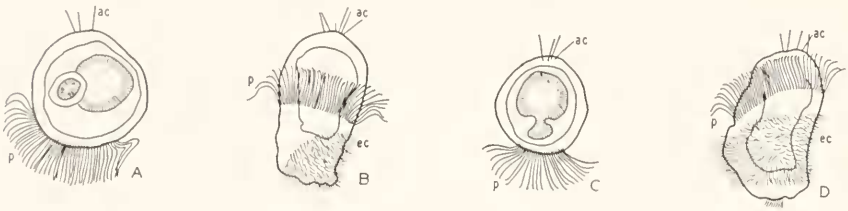


Fig. 2. Larvæ from isolated blastomeres. *A*. *E-PL1* larva, seventy-one hours; with internal gut. *B*. *E-PL1* larva, fifty hours; with exogastrulated endoderm. *C*. *AB* larva, fifty-one hours; with internal gut. *D*. *AB* larva, fifty-six hours; with exogastrulated endoderm.

the present study—are the apical tuft, the post-trochal bristles, the prototrochal cilia, and the apical cilia. The apical tuft forms at about six hours after fertilization, and persists for approximately twenty hours (Fig. 1, *E*). Before it disappears, there develop at the apical end, a number of stiff cilia; these apical cilia remain throughout larval development. The prototrochal cilia appear at about the same time as the apical tuft and they remain throughout larval development. The post-trochal bristles make their appearance toward the end of the first day of development. They increase in length and number as development progresses (Fig. 1, *F*).

Isolation Experiments

E-PL1.—An egg from which the polar lobe is removed at the trefoil stage is labelled *E-PL1*. The first cleavage of *E-PL1* differs from the normal second cleavage in that no polar lobe is formed. As a result,

the quarter-blastomeres are equal in size. In addition, no lobe is formed at the next division. The larvæ of such eggs differ from the normal larva in that: (1) no apical tuft forms; (2) no post-trochal region (including the bristles) appears; and (3) the prototrochal cilia, although of the same size as in normal larvæ, are at the posterior end of the larval ectoderm (Fig. 2*A*, *B*). Although no apical tuft is present, at approximately twenty-four hours after fertilization the typical non-motile cilia appear.

AB.—The isolated *AB* cleaves without the formation of polar lobes and gives rise to a spherical larva similar to that of *E-PL1*. It lacks the apical tuft and post-trochal bristles. It develops normal prototrochal and apical cilia, although the former are situated at the posterior end of the larval ectoderm (Fig. 2*C*, *D*).

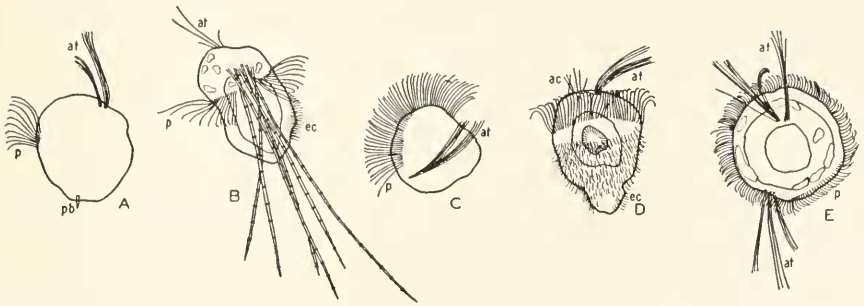


FIG. 3. Larvæ from isolated blastomeres. *A*. *CD* larva, twenty-five hours. *B*. *CD* larva, eighty-five hours. *C*. *CD-PL2* larva, fifty-six hours. *D*. *E-PL2* larva, forty-five hours. (On the next day, the apical tuft was no longer present.) *E*. *E-PL2* larva, eighteen hours. It is exceptional in that it possesses two apical tufts.

CD.—During the first cleavage of *CD* there is formed a polar lobe of the same size as the normal second lobe. After the division, it flows into the *D* cell. At the second cleavage, another, smaller lobe forms from the *D* cell. This lobe has the variable character of the normal third lobe. The early *CD* larvæ appear to be quite normal—the prototrochal cilia are in their usual position and the typical apical tufts are formed (Fig. 3, *A*). However, since the cilia and tuft are of the normal size, they are, proportionately, too large for these reduced larvæ. Later, the paired bristles are formed. However, no apical cilia develop, and in many instances (17 out of 29), the apical tuft does not disappear when it does in controls (Fig. 3, *B*).

CD-PL2.—If during the course of the first cleavage of the *CD* blastomere, the polar lobe is removed, the next cleavage occurs without the formation of a lobe. The *CD-PL2* larva possesses a typical apical

tuft but lacks the post-trochal structures as well as the apical cilia (Fig. 3, *C*).

E-PL2.—It is with difficulty that the second polar lobe is removed from a whole egg. In each of the three larvæ obtained, normal apical tufts and prototrochal cilia develop. (One larva possesses *two* apical tufts—Fig. 3, *E*.) The older larvæ show no post-trochal structures, but do develop apical cilia (Fig. 3, *D*).

BC.—Before the cells have shifted their position after the second cleavage, it is possible to divide them so that *BC* is separated from *AD*. The *BC* combination forms no polar lobe and gives rise to a larva which possesses the typical apical tuft and prototrochal cilia, and a few apical cilia. It lacks completely the post-trochal region (Fig. 4, *B*).

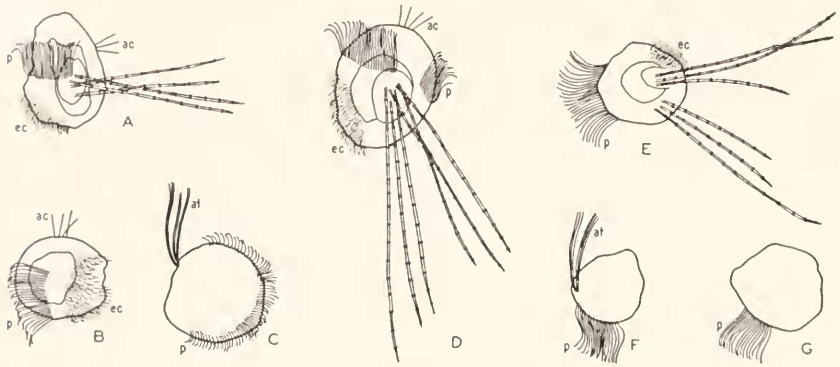


FIG. 4. Larvæ from isolated blastomeres. *A*. *AD* larva, forty-nine hours. *B*. *BC* larva, fifty hours. *C*. *ABC* larva, twenty-six hours. *D*. *ABD* larva, sixty-six hours. *E*. *D* larva, forty-seven hours. *F*. *C* larva, thirty-three hours. *G*. *D-PL3* larva, forty-one hours.

AD.—At the first cleavage of *AD*, a small lobe, which has the variable character of the normal third lobe, forms in the *D* cell. The larva developing from this combination of cells forms no apical tuft. It possesses normal prototrochal cilia and the typical post-trochal region from which extend the bristles. Apical cilia are also present (Fig. 4, *A*).

ABC.—By destroying the *D* cell at the completion of the second cleavage, *ABC* combinations are obtained. No polar lobe is formed at the first cleavage. The larva which develops possesses the typical apical tuft and apical cilia. However, no post-trochal bristles are produced (Fig. 4, *C*).

ABD.—Destruction of the *C* cell leaves the *ABD* blastomeres. At the next cleavage, the *D* cell forms a small polar lobe. The *ABD*

larvæ develop normal prototrochal cilia, but no apical tufts. Later in development, apical cilia and post-trochal bristles appear (Fig. 4, *D*).

C.—No polar lobes are formed during the cleavage of the *C* blastomere. The larva developing from the *C* cell develops typical prototrochal cilia and apical tuft. But neither post-trochal structures nor apical cilia are formed (Fig. 4, *F*).

D.—During the first cleavage of the *D* cell, the small variable lobe appears and passes into the macromere, 1*D*. The *D* larva has typical prototrochal cilia and post-trochal bristles. But it develops no apical tuft and no apical cilia (Fig. 4, *E*).

D-PL3.—In some cases, the lobe formed by the *D* cell constricts sufficiently so that it may be removed. The larva developing from

TABLE I

Summary of larvæ obtained from isolation experiments.

	Number Operated	Number Surviving	Apical Tufts			Post-trochal Bristles		Prototrochal Cilia			Apical Cilia		
			+	−	?	+	−	+	−	?	+	−	?
E-PL1	30	21	0	21		0	11	21	0		14	0	
AB	36	25	0	24		0	18	23	0	1	11	6	1
CD	56	47	37	1	9	32	1	42	0	5	1	26	6
CD-PL2	8	5	5	0		0	4	5	0		0	3	1
E-PL2	3	3	3	0		0	2	3	0		2	0	
BC	10	8	6	2		1	5	8	0		6	0	
AD	6	3	0	3		3	0	3	0		2	0	1
ABC	10	8	7	0	1	0	5	8	0		4	1	
ABD	7	5	0	5		4	0	5	0		4	0	
C	17	11	7	3	1	0	7	11	0		0	7	
D	23	15	0	12	3	9	4	15	0		0	9	4
D-PL3	7	5	0	5		0	3	5	0		0	3	
	213	156											

the *D-PL3* lacks the post-trochal structures as well as the apical tuft and apical cilia (Fig. 4, *G*).

Table I summarizes the isolation experiments. All combinations of cells develop prototrochal cilia. But only those combinations which include the substance of the *C* cell (earlier found in the first polar lobe) develop apical tufts; only those which have the 1*D* cell materials (and previously found in the three polar lobes) form the post-trochal region; and the apical cilia develop only when the *A* or *B* cell is present. Three exceptions, a *BC* larva with bristles, a *CD* larva with apical cilia, and an *E-PL2* larva with two apical tufts, will be discussed later.

Behavior of Isolated Polar Lobes

The changes in form of isolated polar lobes have been described in *Dentalium*, by Wilson (1904*b*) and in *Ilyanassa*, by Morgan (1933, 1935). In *Dentalium*, the isolated lobe usually constricts periodically to form lobe-like structures by a process which simulates the formation of polar lobes in the whole egg. There are three such constrictions, and they occur at approximately the same time as do the cleavages of the whole egg. One case is described in which the final constriction gives rise, not to a temporary lobe, but to a distinctly separated portion of cytoplasm. Wilson interprets the first of these changes as the formation of lobes within the isolated polar lobes, at the time when the whole egg normally forms lobes. The final stage he regards as a

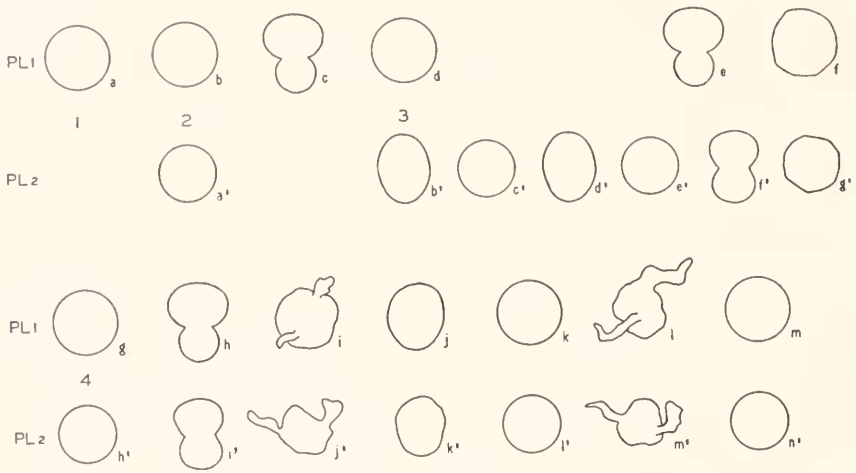


FIG. 5. Form changes in isolated polar lobes. *PL1*, first polar lobe. *PL2*, second polar lobe. Numerals 1–4 indicate the time of the first four cleavages of the whole egg. Explanation in text.

permanent division of the isolated lobe into two at the time of the fourth cleavage of the whole egg, in which the material of the polar lobe no longer forms a temporary polar lobe, but is permanently cut off by a cell division. Morgan argues against a literal interpretation of the form changes in the isolated lobe as lobes. In *Ilyanassa*, he finds that: (1) The changes in the lobe are not strictly synchronous with the cleavage of the whole egg. (2) The constrictions in isolated lobes come and go at least three times, whereas in the whole egg, the lobe would appear only once more. (3) The change in shape does not, strictly speaking, give a reduced picture of the changes in the whole egg; and, (4) the form changes in the lobe resemble more the process of

micromere constriction rather than lobe formation. Therefore, Morgan interprets the later changes of the isolated lobe as related to the constriction of micromeres, and not to the formation of polar lobes.

First Polar Lobe.—Isolated first polar lobes of *Sabellaria* were observed continuously for two to three hours. Although, as both Wilson and Morgan found, the behavior is somewhat variable, most lobes show a remarkable constancy in their changes. Of the 31 isolated lobes studied, 25 were of the type drawn in Fig. 5; the other six were more or less variable.

No change in the shape of the isolated lobe occurs at the time of the first cleavage of *E-PL1*, i.e., at the time of the second cleavage of the whole egg (Fig. 5, *b*). About ten to fifteen minutes after the first cleavage of *E-PL1*, the lobe is deeply constricted to form a definite lobe-like structure (Fig. 5, *c*). At the time of the second cleavage, the lobe is spherical (Fig. 5, *d*), but another deep constriction appears about ten to fifteen minutes later (Fig. 5, *e*). As this second constriction disappears, a slight flattening of the lobe occurs (Fig. 5, *f*). This lasts for a short time, and when *E-PL1* cleaves for the third time, the lobe is again spherical (Fig. 5, *g*). A third constriction forms after the third cleavage of *E-PL1* (Fig. 5, *h*), but before it disappears completely, the entire cell becomes irregular in outline, and long irregular pseudopodia are formed (Fig. 5, *i*). These are later withdrawn (Fig. 5, *j*) and the cell again becomes spherical (Fig. 5, *k*). However, it remains in this condition for only a short time, until the irregular pseudopodia are formed again (Fig. 5, *l*). The extension and retraction of the pseudopodia is not synchronous with cleavage, and continues until the lobe cytolyzes. In several cases, the process was still going on twenty-eight hours after the removal of the lobe; in one case, it was observed up to forty-eight hours after the separation of the lobe.

Second Polar Lobe.—The second polar lobes were removed from five isolated *CD* cells, and their behavior followed continuously for two hours. Four of the five lobes produced fairly deep constrictions twice (Fig. 5). The other produced only the first of the two constrictions; at the time when the second constriction would form, the cell elongated somewhat without constricting. The behavior of the four lobes which formed the two constrictions was fairly uniform. When *CD-PL2* divides for the first time, i.e., at the time of the third cleavage of the whole egg, the isolated lobe elongates slightly (Fig. 5, *b'*) and within a minute is rounded out (Fig. 5, *c'*). A similar elongation forms again, in three of the lobes, in about eight to ten minutes after the first (Fig. 5, *d'*). At about seventeen to eighteen minutes after the first cleavage, the lobe develops a constriction which persists for two

minutes (Fig. 5, *f'*). As the constriction disappears, the lobe flattens slightly (Fig. 5, *g'*). Within a few minutes, the second cleavage of *CD-PL2* occurs. Following the second cleavage a second constriction forms (Fig. 5, *i'*), and as it disappears the cell becomes irregular and gives rise to pseudopodia (Fig. 5, *j'*). The behavior of the pseudopodia is similar to those of isolated first lobes (Fig. 5, *j'–n'*).

The constrictions formed by the isolated first and second polar lobes resemble in appearance the polar lobes formed by the dividing ovum. Although these constrictions do not occur at the same time as the cleavages of the ovum, they must, in some way, be related to events taking place in the egg during division. This is brought out by a comparison of the time elapsing between successive cleavages of the lobeless egg and the constrictions of the isolated lobe, in the twenty-five cases where the first polar lobe was removed and the five in which the second lobe was removed. The average time between the first and second cleavages of *E-PL1* is 20.6 minutes and that between the first and second constrictions of the isolated first lobe 18.6 minutes. The time between the second and third cleavage of *E-PL1* is 24.6 minutes and that between the second and third constriction is 21.2 minutes. For the isolated second lobes, the average time between the two constrictions is 21.8 minutes, while the interval between the first and second division of *CD-PL2* is 21.0 minutes. The changes in the form of the isolated lobes are apparently synchronous with the cleavages of the ovum, except that all events in the isolated lobes are pushed back by a delay in the appearance of the initial constriction. This delay is approximately ten to fifteen minutes for the isolated first lobe and about seventeen to eighteen minutes for the isolated second lobe.

On the basis that the periodic constrictions formed by isolated polar lobes are correlated with cytoplasmic changes occurring in whole eggs at the time of the cleavages in which polar lobes are formed, we would expect the isolated second lobe to form one fewer constriction than the isolated first lobe. The isolated first lobe forms three constrictions; the isolated second lobe two. And, as would be expected, the time elapsing between the first and second constrictions of the isolated second lobe (21.8 minutes) is almost identical with the time between the second and third constrictions of the isolated first lobe (21.2 minutes). However, certain differences between the behavior of the isolated lobes and that of polar lobes of the whole egg must be noted. The whole egg forms two polar lobes after the formation of the first lobe, and one after the second. The isolated first lobe, however, forms three constrictions and the isolated second lobe two.

Although there is a decrease in size of successive lobes in the whole egg, the constrictions of the isolated lobe are all of approximately the same size. But the facts to be emphasized are: (1) that there reside in the isolated polar lobes materials which take part, independently of the nucleus or the mitotic apparatus, in reactions affecting the tension at the surface of the cell, and (2) that these reactions in isolated lobes occur synchronously (if we discount the initial delay) with events in the whole egg, or in the egg from which the polar lobe has been removed.

TABLE II

Differentiation of larvæ after transplantation of polar lobes and blastomeres.

Type of Operation	Number Operated	Number Survived	Larvæ with: 0 Apical Tuft	1 Apical Tuft	2 Apical Tufts	Larvæ with: 0 Sets of Bristles	1 Set of Bristles	2 Sets of Bristles	Larvæ with: 0 Groups of Apical Cilia	1 Group of Apical Cilia	2 Groups of Apical Cilia
A. Polar lobe transplants											
Transplant *Host*											
PL1 Whole egg	26	23	7	16	0	8	14	0	7	15	0
AB	35	32	32	0	0	20	0	0	5	14	0
E-PL1	15	14	14	0	0	10	0	0	3	7	0
PL2 Whole egg	6	5	0	5	0	2	2	0	1	3	0
AB	6	6	6	0	0	3	0	0	0	3	0
B. Transplantation of blastomeres to whole egg											
Transplant											
CD	9	9	0	2	7	0	1	6	1	5	0
C	10	10	0	4	6	0	8	0	2	6	0
D	16	14	2	10	0	0	4	10	2	12	0
AB	11	11	2	9	0	2	8	0	1	4	5
C. Transplantation of blastomeres to *E-PL1*											
Transplant											
CD	16	16	2	11	0	2	12	0	5	9	0
C	20	18	6	12	0	14	0	0	5	8	0
D	15	15	15	0	0	0	13	0	4	9	0
CD-PL2	4	3	1	2	0	2	0	0	0	2	0
AB	12	11	11	0	0	9	0	0	0	3	6
Whole egg	3	3	1	2	0	0	3	0	0	1	2
D. Fusions of blastomeres											
Combination											
CD, CD	20	19	0	11	8	2	2	11	15	0	0
CD, D	9	8	0	7	0	1	1	5	7	0	0
CD, C	6	4	1	2	1	0	2	0	2	0	0
C, C	4	3	0	2	0	2	0	0	2	0	0
AB, AB	12	10	10	0	0	8	0	0	1	2	5
3 *AB*'s	3	2	2	0	0	1	0	0	0	0	1
5 *AB*'s	1	1	1	0	0	1	0	0	Three groups of cilia		

TRANSPLANTATION EXPERIMENTS

Transplantation of Polar Lobes

Transplants of first and second polar lobes were made to whole eggs at the trefoil, two-cell, and four-cell stages, to *AB* blastomeres, and to *E-PL1*. The lobes were placed at the animal pole, at the vegetal pole, and at the equator of the dividing egg. Although

fused to the blastomeres, the lobes go through essentially the same form changes as do isolated lobes. When cleavage progresses, the blastomeres may grow over and completely enclose the lobe, or the lobe may remain at one end of the larva. The lobe is still part of the larva when the cilia appear (Fig. 6*A*, *C*). Prototrochal cilia form in all larvæ, and the apical tufts develop only in those which include *C* cells (Table II, *A* and Fig. 6). At about fifteen hours after fertilization, the lobe, more or less completely cytolyzed, is extruded from the larva (Fig. 6, *D*). This is generally followed by a cytolysis of a portion of the embryo, especially in those cases where the lobe was deeply embedded within the embryo. Thus, only 59 out of the 80 larvæ

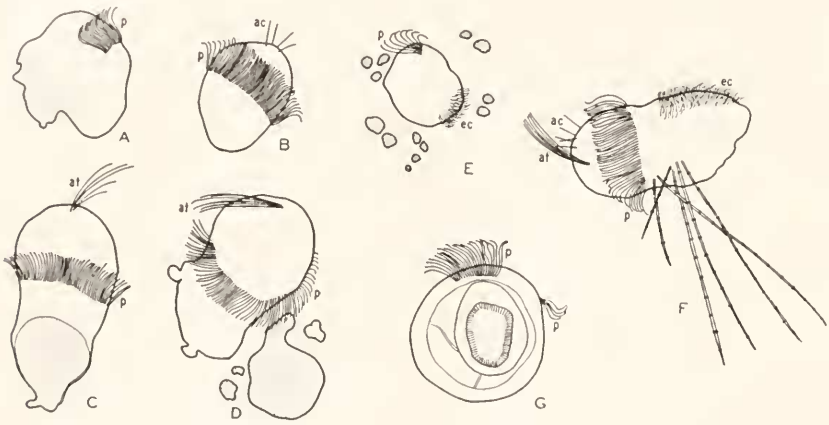


FIG. 6. Differentiation of larvæ after transplantation of polar lobes. *A*. *AB* plus *PLI* larva, 11½ hours. The lobe (stippled) is within the larva. *B*. *AB* plus *PLI* larva, 24 hours. The larva has moved away from the cytolyzed spheres of the ejected lobe. *C*. *E* plus *PLI* larva, 13 hours. The lobe (stippled) is within the larva. *D*. *E* plus *PLI* larva, eighteen hours. The lobe has been ejected. *E*. *AB* plus *PLI* larva, 45 hours. The ejected lobe has broken up into small spheres. *F*. *E* plus *PLI* larva, 26 hours. *G*. *E-PLI* larva, 50 hours. The first polar lobe was in contact with the dividing egg for five hours, at the end of which time it was removed.

survived beyond twenty-four hours. Where the larva is not greatly damaged by the loss of the lobe, differentiation progresses normally. The *AB* and *E-PLI* larvæ form apical cilia but no post-trochal regions (Fig. 6, *B*). The larvæ produced by entire eggs lose their apical tufts when apical cilia appear, and they develop post-trochal regions with typical bristles (Fig. 6, *F*).

In many cases, the polar lobe was stained heavily with Nile Blue sulphate, before being transplanted. The dye diffused from the lobe into the adjacent cells, so that the larval tissues acquired a pronounced blue coloration.

To avoid possible effects of the lobe cytolysis on the differentiation of the larva, the lobe was, in fifteen cases, allowed to remain fused to the dividing cells only until the embryo began to show signs of movement, when it was removed. The contact is long enough so that the blue color of the dye diffuses from the lobe into adjacent cells. The larvæ which develop from such cells are in no essential way altered by contact with the lobe (Fig. 6, *G*).

The presence of the polar lobe, fused to the larva for five hours (when it is removed), or within the larva for eleven hours (at which time it is ejected), does not induce the formation of larval structures.

Transplantation of Blastomeres to Whole Eggs

Table II, *B* summarizes the types of transplants made to whole eggs, at the trefoil, two-cell, and four-cell stages. The transplants

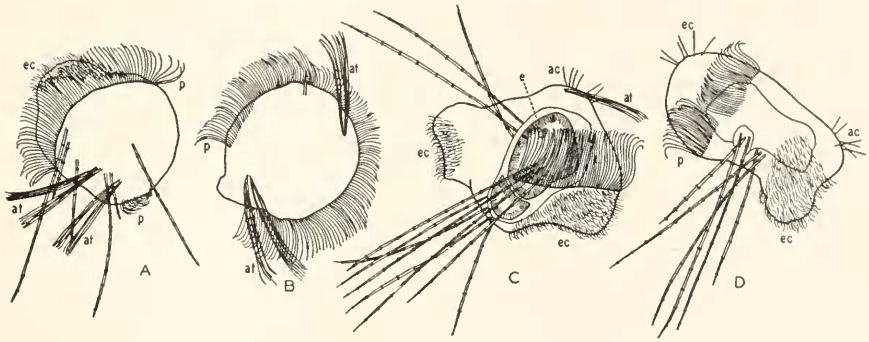


FIG. 7. Differentiation of larvæ after transplantation of blastomeres to whole eggs. *A*. *E* plus *CD* larva, 27 hours. *B*. *E* plus *C* larva, 24 hours. *C*. *E* plus *D* larva, 79 hours. *D*. *E* plus *AB* larva, 40 hours.

are in some cases stained with Nile Blue sulphate before being fused to the host. It is possible to follow the stained region through the first day of development, but beyond this time the dye is not visible. The orientation is varied, but no correlation between any particular orientation and type of development is found.

The differentiation of the transplanted *AB*, *CD*, *C*, or *D* blastomeres does not bring about the development of additional structures in the host; those structures which are duplicated in the larva arise from the self-differentiation of the transplanted cells. Seven of the nine *E* plus *CD* larvæ show two apical tufts; six (out of the seven surviving) show two sets of post-trochal bristles (Fig. 7, *A*). In six of the ten *E* plus *C* larvæ, two apical tufts are visible (Fig. 7, *B*). Ten of the fourteen *E* plus *D* larvæ possess two sets of bristles (Fig. 7, *C*). The *E* plus *AB* larvæ show no duplication of either the apical tuft or

bristles, but five of the ten possess two distinct groups of apical cilia (Fig. 7, *D*).

Transplantation of Blastomeres to E-PL1

The polar lobes are removed from eggs at the trefoil stage, and in their place are put half- or quarter-blastomeres, or whole eggs. In other experiments, transplants are placed at the animal pole, or to the side of *E-PL1*. As in previous operations, the transplanted cells are first stained, either *in toto*, or locally, to mark their polarity. The resultant larvæ are summarized in Table II, *C* and a few of the various kinds are shown in Fig. 8. In all larvæ, the host cells give rise to

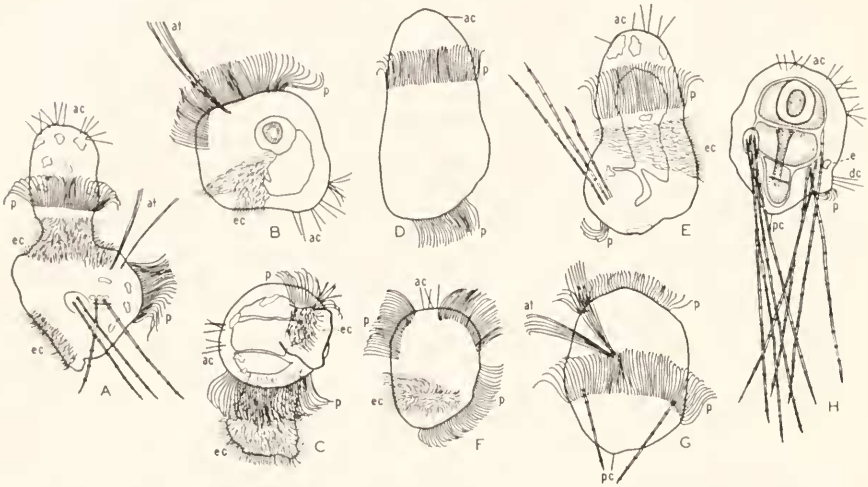


FIG. 8. Differentiation of larvæ after transplantation of blastomeres to *E-PL1*. *A*. (*E-PL1*) plus *CD* larva, 43 hours. *B*. (*E-PL1*) plus *C* larva, 30 hours. *C*. (*E-PL1*) plus *C* larva, 48 hours. *D*. (*E-PL1*) plus *D* larva, 17 hours. *E*. Same larva as shown in *D*, 43 hours. *F*. (*E-PL1*) plus *AB* larva, 54 hours. *G*. (*E-PL1*) plus *E* larva, 25 hours. *H*. Same larva as shown in *G*, 91 hours.

prototrochal cilia, and in at least 36 of 55, they form apical cilia. They form no apical tuft and no post-trochal bristles. The apical tufts, post-trochal bristles, or supernumerary prototrochal and apical cilia which are present in the larvæ arise only through the self-differentiation of the transplant.

Fusions of CD, C, and D Blastomeres

Thirty-four operations involving combinations of *CD* cells with *CD*, *D*, and *C* blastomeres, and fusions of two *C* cells were performed (Table II, *D* and Fig. 9). Among the 19 larvæ with the *CD*, *CD* combination, 8 show two apical tufts; in 11, only one tuft is distinctly

visible. Of the 14 larvæ surviving beyond the first day, 2 fail to develop post-trochal bristles, 2 form one set of bristles, and 11 develop two distinct sets. There are 7 larvæ with the *CD*, *D* combination. Each of these larvæ produces one apical tuft, and 5 of the 7 show two sets of post-trochal bristles. One larva forms one set of bristles and another forms none. Four larvæ of the *CD*, *C* constitution show the following: two apical tufts, 1; one apical tuft, 2; no apical tuft, 1. Two survive long enough to develop bristles; in each there is one set. Only two *C* plus *C* larvæ survive; each possesses only one apical tuft and none forms post-trochal bristles except those which include *D* cells.

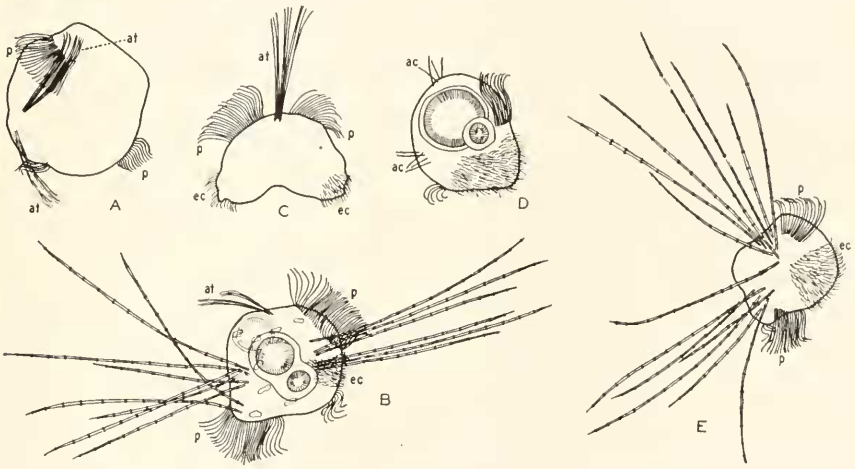


FIG. 9. Differentiation of larvæ arising from fusions of blastomeres. *A*. *CD* plus *CD* larva, 13 hours. *B*. Same larva as shown in *A*, 59 hours. *C*. *C* plus *C* larva, 28 hours. *E*. *CD* plus *D* larva, 55 hours. *D*. *AB* plus *AB* larva, 50 hours.

Fusions of AB Blastomeres

At the time of the first cleavage of isolated *AB* cells, various combinations are effected. Of the larvæ surviving, ten come from fusions of two *AB* cells, two from three *AB*'s, and one from five. Five of seven of the *AB*, *AB* larvæ show two sets of apical cilia, and two show one set; in the single surviving larva from the combination of three *AB*'s, two sets of apical cilia are seen; in the larva from the fusion of the five *AB*'s three distinct sets of apical cilia are visible. Although there is a superabundance of cellular material, none of the larvæ develops an apical tuft and none develops a post-trochal bristle (Table II, *D* and Fig. 9, *D*).

Fusions of Whole Eggs

Two eggs are fused at the trefoil stage or at the two-cell stage. The orientation of the two eggs with respect to each other is varied as follows: (1) the animal-vegetal axes of the ova remain parallel, but the eggs are rotated to different degrees; (2) one of the two eggs is inverted so that the fusion occurs at the animal poles or at the vegetal poles of the two eggs, with the rotation of the eggs varied as in (1).

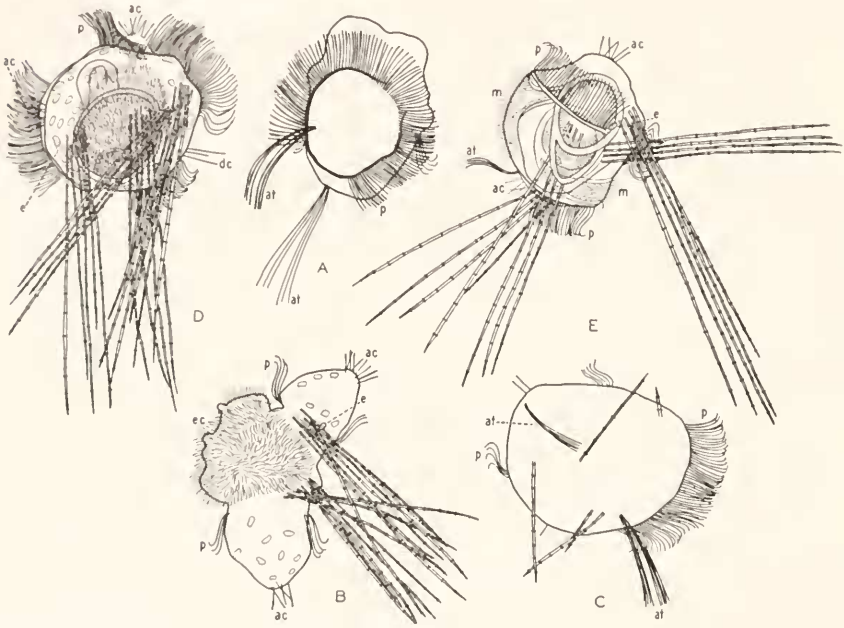


FIG. 10. Differentiation of larvæ arising from fusions of two eggs. *A*. Larva, 23 hours. *B*. Same larva as shown in *A*, 52 hours. *C*. Another larva, 27 hours. *D*. Another larva, 49 hours, with one internal gut. On the outer surface is an area of ciliated cells, devoid of chromatophores. *E*. Another larva, 55 hours, with two internal guts.

Of the twenty-five fusions effected, 23 larvæ are alive at the end of twenty hours of development, and 19 at the end of thirty-five hours. Of the 23, 2 larvæ show no apical tuft, 7 show one tuft, and 11 show two tufts. Among the 19 larvæ, the post-trochal bristles fail to form in one case, 4 larvæ have one set of bristles, and 14 have two sets; the apical cilia are not visible in 2 larvæ, 5 show one group of cilia, and 12 show two distinct groups. In none of the larvæ are there more than two apical tufts, or more than two sets of post-trochal bristles, or more

than two groups of apical cilia. Figure 10 includes three advanced larvæ of different types. In one type (Fig. 10, *B*), the endoderm cells of both eggs have been exogastrulated. In another (Fig. 10, *E*), there are two distinct internal guts, of fairly normal structure. In a third type (Fig. 10, *D*), only one internal gut is found. This gut has the typical tripartite character and is no larger than the normal gut. On the outer surface of the larva, a wide, delimited, area is devoid of the chromatophores characteristic of the ectoderm, and the cells of this area are covered with the rapidly-moving cilia, characteristic of the gut cells. The constitution of this area is therefore interpreted as the endoderm cells of one of the eggs. Instead of giving rise to a gut, these cells have become part of the outer covering of the larva; but in spite of their new location, they continue to differentiate as they would normally.

In two instances, three eggs are fused at the two-cell stage. The resultant larvæ develop three sets of post-trochal bristles. In one case, four eggs are fused at the two-cell stage, and the larva develops four sets of bristles.

Discussion of Results

By means of isolation experiments, the independent developmental capacities of early blastomeres of *Sabellaria vulgaris* were determined. These experiments included the usual separation of half- and quarter-blastomeres, and, in addition, the separation of other combinations of cells (*AD*, *BC*, *ABD*, and *ABC*). Also, the effect of the removal of three polar lobes was studied. Table I classifies the 156 surviving larvæ, with respect to the presence or absence of the prototroch, apical tuft, post-trochal bristles, and apical cilia. Prototrochal cilia were present in all larvæ. The apical tuft formed only in those larvæ which included the first polar lobe and the *C* cell. The post-trochal bristles developed only when the three polar lobes and the 1*D* cell were present. The differentiation of apical cilia occurred only when either the *A* or the *B* cell was present. Two of the larvæ were exceptions to this conclusion: one *CD* larva, which developed apical cilia, and one *BC* larva, which developed post-trochal bristles. A third larva, of the *E-PL2* type, formed two apical tufts instead of one.

Having determined the fate of the blastomeres when isolated, the behavior of these same cells when placed in contact with each other atypically, or with isolated polar lobes, was investigated. The twenty-two types of transplantation experiments, including a total of 247 larvæ, are summarized in Table II. Not a single case is found in which the transplant had induced the formation of any particular structure

in the host. In any combination of blastomeres, apical tufts develop only when *C* cells are present, post-trochal bristles are dependent upon the presence of *D* cells, and apical cilia form only when either the *A* or *B* cell is included.

In Table III, the larvæ are classified on a different basis. The number of *C*, *D*, and *A* or *B* cells included in the makeup of the individuals are compared with the number of apical tufts, post-trochal structures, and apical cilia. In general, the number of apical tufts is

TABLE III

Classification of larvæ developing from transplantation experiments.

Number of *C* Cells	Total No. of Larvæ	Number of Larvæ with		
		0 Apical Tufts	1 Apical Tuft	2 Apical Tufts
0	88	88	0	0
1	95	21	74	0
2	64	3	28	33

Number of *D* Cells *	Total No. of Larvæ	Number of Larvæ with		
		0 Sets of Bristles	1 Set of Bristles	2 Sets of Bristles
0	69	69	0	0
1	63	14	49	0
2	62	4	12	46

Number of *A* (or *B*) Cells	Total No. of Larvæ	Number of Larvæ with		
		0 Apical Cilia	1 Group of Apical Cilia	2 Groups of Apical Cilia
0	26	26	0	0
1	128	35	93	0
2	49	4	15	30

* Two larvæ had 3 *D* cells; both had 3 sets of bristles. One larva had 4 *D* cells; it had 4 sets of bristles.

determined by the number of *C* cells present, the number of sets of post-trochal bristles by the number of *D* cells, and the number of groups of apical cilia by the number of *A* (or *B*) cells. The absence of a higher correlation, particularly in the cases of the apical tuft and apical cilia, may possibly be due to several reasons: (1) The larvæ are actively swimming and, especially in small, healthy individuals, such minute structures may be overlooked. (The post-trochal bristles are much more readily seen. The correlation is higher with this structure than

with either of the other two.) (2) Some of the operated individuals may have been injured and the failure to form a particular structure may be a manifestation of their reduced vitality. (3) Actively swimming larvæ may have some of their cells torn away by the surface at the edge of the drop of water. It is in many cases not possible to determine whether this has occurred. (4) The mechanical effects of the neighboring tissues may prevent the cells from giving rise to the particular structure. If true, this effect might possibly be conceived as a kind of regulatory process.

The most significant feature of the transplantation experiments is that in no instance is the number of apical tufts greater than the number of *C* cells, nor the number of post-trochal regions greater than the number of *D* cells, nor the number of groups of apical cilia greater than the number of *A* (or *B*) cells.

Of special interest are several larvæ which developed from a fusion of two eggs at the two-cell stage. In these larvæ (Fig. 10, *D*), there is present a single gut, of normal size and typical tripartite structure. In one of the two eggs, the cells whose prospective value is gut endoderm have been incorporated into the outer covering of the larva. Although "ectodermal" in the sense of location, these cells continue to differentiate into endoderm, i.e., they do not develop chromatophores and they become ciliated on their outer surface. The self-differentiation of the endoderm in this position indicates, as does the development of exogastrulæ (Novikoff, 1938), the complete independence of endodermal and ectodermal differentiation in *Sabellaria vulgaris*.

Since the polar lobe, as well as any of the quarter- or half-blastomeres, does not affect the differentiation of any cell through contact with that cell, it is not possible to consider the polar lobe an "organizer," in the sense of Spemann (cf. Wilson, 1929, pp. 202–205, and Huxley and deBeer, 1934, pp. 171–172). The experiments of Tyler (1930) have demonstrated that when the first cleavage of the *Chætopterus* egg is made equal—either through the retraction of the polar lobe into the smaller, *AB*, blastomere, or through the cleavage furrow dividing both the egg and the polar lobe equally—then the two half-blastomeres are totipotent. In both types of equal cleaving eggs, the *AB* blastomere receives polar lobe material. If allowed to develop *in toto*, such eggs produce double monsters of the cruciata type. (At the second cleavage, two very small polar lobes may be formed.) When separated, each half-blastomere produces a fairly normal larva. There is in reality no "*AB*" cell; each cell behaves like a *CD*. But there is no evidence that the substance, whose altered distribution

changes the prospective value of the cell from that of an *AB* to that of a *CD* cell, is located within the polar lobe. The fact that a double embryo is produced when the polar lobe goes in its entirety into the *AB*, i.e., that the *CD* can produce an embryo without the materials of the polar lobe, indicates the complexity of the situation. That the vegetal hemisphere of the molluscan or annelid egg possesses a particular substance at the time of the first cleavage is well established by the work on *Ilyanassa* (Crampton, 1896), on *Dentalium* (Wilson, 1904*b*), on *Tubifex* (Penners, 1924, 1926), and on *Sabellaria* (Hatt, 1932, and the present paper). In *Tubifex*, if the first cleavage of the whole egg or the *CD* blastomere is made to take place equally instead of unequally (through heat or lack of oxygen)—and the pole-plasms are distributed equally to the two cells—double cruciata monsters are produced (Penners, 1924). A single case was observed in *Sabellaria* in which the first two blastomeres were of equal size. When isolated, each of the blastomeres produced polar lobes at the next two cleavages, and each gave rise to a larva possessing an apical tuft. The fact that each cell forms polar lobes and that the two cells are of equal size indicates that each cell probably received materials from the first polar lobe. It would, then, appear that some substance present in the first polar lobe *does* have the ability to change the course of development of a cell, but that this substance does not act by contact with a cell; it must become a part of the cell. Normally, only the *CD* cell develops an apical tuft since the materials of the lobe flow only into that cell. The exceptional production of apical cilia by a *CD* cell and the appearance of post-trochal bristles in a *BC* combination, mentioned earlier, may be due to a deviation in either of the first two cleavage furrows, with a resultant unusual allocation of materials. The doubling of the apical tuft in the *E-PL2* larva may also be due to an unusual pattern, in which the materials giving rise to apical tufts are separated into different cells.

In the so-called regulative eggs, it is in many cases possible to alter the course of differentiation of a cell by transplanting the cell to a new position in the developing embryo. By varying the stage at which the operation is performed, the time of determination of a structure may be ascertained. Due to the scarcity of similar experiments on mosaic eggs, relatively little information is available concerning the effect of one part of an embryo on another during the course of development, or the existence of inducing, or organizing, regions in these eggs. Penners (1926, 1934) destroyed varying numbers of mesodermal and ectodermal teloblasts of *Tubifex*, at different stages, to test (1) the inter-dependence of ectoderm and mesoderm during development, and

(2) the inducing capacity of the teloblasts. He found that the ectoderm and mesoderm show complete independent differentiation, except for a slight influence of the development of one upon the form and upon the rate of development of the other. The fact that following the destruction of the teloblasts the embryos continue to develop normally indicates that the teloblasts are not organizing centers. Hörstadius (1937*b*) combined various quartets of blastomeres of the sixteen-cell stage in *Cerebratulus;* he found no effect of one layer upon the differentiation of the others. These results are in agreement with those of the present investigation, in which the polar lobe, half-, and quarter- blastomeres are shown to be ineffective in directing the development of *Sabellaria*.

However, in another egg which was thought to be mosaic, the egg of the ascidian, effects of one cell upon the development of the other have been reported. Tung (1934) found that some factor outside the brain is responsible for the formation of the sense organ, in *Ascidiella*. Also, Tung found indications that the adhesive organ is induced. A recent paper by Rose (1937) reports that, similarly, in the egg of *Styela*, the eye spot is induced by the gray macromeres.

Summary

1. Isolation experiments on the egg of *Sabellaria vulgaris* demonstrate that the formation of the apical tuft in partial larvæ is dependent upon the presence of the first polar lobe and the *C* cell; that the post-trochal region develops only when the three polar lobes and the 1*D* cell are present; and that apical cilia form only if the *A* or *B* cell is included.

2. Form changes in isolated first and second polar lobes are described. The early changes are synchronous with the cleavages of the ovum, except that all events in the isolated lobe are delayed.

3. The results of the following transplantation experiments are reported: (*a*) Transplantation of polar lobes. (*b*) Transplantation of blastomeres to the whole egg. (*c*) Transplantation of blastomeres to *E-PL1*. (*d*) Fusions of half- and quarter-blastomeres. (*e*) Fusions of two eggs. In all combinations, complete self-differentiation of individual blastomeres occurs. Apical tufts develop only when *C* cells are present, post-trochal bristles are dependent upon the presence of *D* cells, and apical cilia form only when either the *A* or *B* cell is included.

4. The results of this investigation are compared with those from experiments on other mosaic eggs.

It is with pleasure that the writer expresses his gratitude to Pro-

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